Interaction forces drive the environmental transmission of pathogenic protozoa RUNNING TITLE: Protozoa-environment interaction forces

Aurélien Dumètre, Laurence Voutquenne-Nazabadioko, Pierre-Henri Puech, Jeanne Hohweyer, Nadine Azas, Isabelle Villena

Author edited document

Interaction forces drive the environmental transmission of pathogenic protozoa

***RUNNING TITLE*: Protozoa-environment interaction forces**

Aurélien Dumètre,[1*] Dominique Aubert,[2] Pierre-Henri Puech,[3] Jeanne Hohweyer,[2] Nadine Azas,[1] and Isabelle Villena[2]

[1] Aix-Marseille Université, UMR MD3 Relations Hôte-Parasites, Pharmacologie et Thérapeutique, Faculté de Pharmacie, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 05, France.

[2] Université de Reims Champagne-Ardenne, Laboratoire de Parasitologie-Mycologie, EA 3800, Faculté de Médecine, IFR 53, 51 rue Cognacq-Jay, 51096 Reims, France.

[3] INSERM U600 / CNRS UMR6212, Laboratoire Adhésion Cellulaire et Inflammation, Faculté des Sciences, 163 Avenue de Luminy, 13288 Marseille Cedex 09, France.

* Corresponding author: Aurélien Dumètre

Telephone: +33 4 91 83 55 44 / Fax: +33 4 91 83 55 37

E-mail: aurelien.dumetre@univmed.fr

Full postal address:

UMR-MD3, Laboratoire de Parasitologie,

Faculté de Pharmacie,

27 Bd Jean Moulin

13385, Marseille Cedex 05, France.

ABSTRACT

The protozoan parasites *Giardia duodenalis*, *Cryptosporidium* spp. and *Toxoplasma gondii* are environment-resistant pathogens that pose significant risks to public health worldwide. Their environmental transmission is closely governed by the physicochemical properties of their cysts and oocysts respectively, allowing their transport, retention and survival for months in water, soil, vegetables and mollusks, which are the main reservoirs for human infection. Importantly, the cyst/oocyst wall plays a key role in that regard by exhibiting a complex polymeric coverage that determines the charge and hydrophobic characteristics of parasites' surface. Interaction forces between parasites and other environmental particles may be, in a first approximation, evaluated following the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory of colloidal stability. However, due to the molecular topography and nano- to micro- structure of the cyst/oocyst surface, non-DVLO hydrophobic together with additional steric attractive/repulsive forces may play a pivotal role in controlling the parasite behavior when submitted to various external conditions. Here, we review several parameters that enhance or hinder the adhesion of parasites to other particles and surfaces, and address the role of fast-emerging techniques for mapping the cyst/oocyst surface e.g. by measuring their topology and the generated interaction forces at the nano- to micro- scale. We discuss why characterizing these interactions could be a crucial step for managing the environmental matrices at risk of microbial pollution.

INTRODUCTION

The protozoan parasites *Giardia duodenalis*, *Cryptosporidium* spp. and *Toxoplasma gondii* are major pathogens able to survive in contrasting aquatic or terrestrial environments in order to infect a wide range of vertebrate hosts occupying very different ecological niches (34). Definitely, their environmental transmission poses significant risks to human health. *Giardia* cysts and *Cryptosporidium* and *Toxoplasma* oocysts are typically acquired by consuming waters or foods that are inadequately treated to kill or to remove the parasites (29, 59-61, 95, 96). Resulting infections are among the most prevalent parasitic diseases worldwide. *Giardia* and *Cryptosporidium* are responsible for gastrointestinal diseases causing mild to severe diarrhea (11, 109), whereas *Toxoplasma* infections may lead to threatening birth defects, severe neurological and ocular diseases depending on the parasite and host genetic backgrounds (77, 85).

The environmental impact of these parasites greatly is closely related to their extended survival to contrasting climatic conditions and disinfection processes (5, 60, 63), and in their ability to interact with other organic or non-organic particles. This latter phenomenon governs their transport, retention/release and survival from land to sea (1, 2, 82, 98-100). The cyst/oocyst wall plays a key role by forming a highly resistant barrier to a large set of physicochemical stressors and by, at the same time, exhibiting surface properties involved in parasite-particle interactions (7, 23, 30, 101). Though the biochemical composition and molecular architecture of their respective outer wall greatly differ (14, 47, 57, 64, 76, 89, 91) (Figure 1), those three parasites could present similar surface interactions with their surrounding world due to their bio-physical features (Table 1). Such interactions may be described in a first approximation using prediction models of colloidal stability and attractive/repulsive forces (110). Importantly, interaction forces depend on the chemistry and topography of the macromolecules at the parasite surface, their hydrophobicity and electric

charge, and on external physicochemical conditions, such as ionic composition of the surrounding media and organic contaminations, which can also contribute to promote or hinder parasite adhesion (21, 23, 56, 67-69, 74, 110). First measuring interaction forces and understanding their origin, then controlling them appear therefore critical in regulating the fate of parasites in the different aquatic and telluric environments, and consequently their transmission to animals and humans.

In this review, we describe the different parameters contributing to the interactions between the environmentally resistant stages of *Giardia*, *Cryptosporidium* and *Toxoplasma* and other particles, and point out the importance of an accurate characterization of underlying forces to better predict parasites distribution through the environment and therefore prevent their transmission to humans.

SURFACE CHEMISTRY OF THE CYST/OOCYST WALL

The biochemical nature of the macromolecules at the cyst/oocyst surface inherently contributes to the interactions between the parasites and their environment. Thanks to the combination of powerful imaging analysis techniques such as confocal laser-scanning microscopy and immunoelectron microscopy, and chemical methods e.g. gas chromatography-mass spectrometry, the list of described macromolecules composing the cyst/oocyst wall surface have been recently extended (57, 64, 76, 91).

The quadranucleate cysts of *Giardia duodenalis* form in the intestinal lumen of the infected host following a complex multifactorial process (70). The cyst wall is 300 to 500 nm thick and mainly consists in a surface filamentous layer (Figure 1) and is built with materials that originate from encystation-specific secretory vesicles appearing in the encysting parasites (44, 64). The biochemical composition and structural arrangement of the filamentous layer consist in a dense network of curled fibrils of *N*-acetylgalactosamine measuring ~10 nm in diameter

97(13). These fibrils are closely associated with certain wall proteins called cyst wall proteins
98(CWP) (13). Four major proteins have been identified in the cyst wall. They include the
99CWP1-3 proteins, which harbor *N*-terminal leucine-rich repeats together with a C-terminal
100cysteine-rich region, and a fourth belonging to the family of cysteine-rich non-variant-specific
101surface proteins of *Giardia* (25). Aside, an epidermal growth factor-like cyst protein has been
102shown to be involved in the cyst wall formation, in partnership with the non-variant-specific
103surface protein (16). The thick filamentous outer layer of the cyst wall has been shown to be
104fully impermeable to water-soluble substances, enhancing the survival of cysts in water and
105resistance to disinfectants (5).

106The four infective sporozoites of *Cryptosporidium* are protected by a complex multilayer wall
107of 50-70 nm thickness that forms while the oocyst develops in the intestinal cells of the
108infected hosts (47, 57, 89). The oocyst wall of *Cryptosporidium* is mainly built with materials
109released sequentially by different subsets of specific organelles found in the cytoplasm of the
110fertilized macrogamete, the so-called wall forming bodies (103). Current proposed data show
111an inner layer of glycoproteins and a central lipid-protein layer covered by an outer glucose-
112rich glycocalyx (12, 57, 87) (Figure 1). Large molecular weight cysteine-rich proteins, namely
113*Cryptosporidium* oocyst wall proteins (COWPs), are thought to form extensive disulphide
114bridges and, consequently, matrices in the inner layer that chiefly provides the overall
115mechanical strength of the oocyst wall (103, 108). The glycocalyx, decorating the wall
116structure and facing the surrounding media, provides at the same time immunogenicity and
117potential attachment possibilities (57, 87). The outcomes of physical and chemical treatments
118of the oocyst wall indicate that the glycocalyx is delicate and highly susceptible to
119disinfectants such as sodium hypochlorite, and to conservative agents like formalin (43, 47).

120The wall of the sporulated oocyst of *Toxoplasma* encloses two sporocysts containing each
121four infectious sporozoites (36, 104). As a conserved feature among Coccidia, the double-

layered wall of the *Toxoplasma* oocyst forms a highly resistant and impermeable shell (3). Nonetheless, the outer layer can be stripped off easily in a rather easy way using chemical treatments, so the robustness of the oocyst wall appears to be mainly due to its inner layer (76). The ~100 nm thick oocyst wall forms while the oocyst is still housed by the enterocytes of cats, the definitive hosts of the parasite (37). This structure is built with materials released sequentially by different types of wall forming bodies present in the cytoplasm of the macrogamete/early stage oocyst (37). The oocyst wall of *Toxoplasma* (and of related coccidian parasites) is at more than 90% made of proteins, with two identified types so far, namely cysteine- and tyrosine-rich proteins (76) (Figure 1). In *Toxoplasma*, three cysteine-rich oocyst wall proteins (OWP), TgOWP1-3, out of the seven encoded by the parasite genome, have been recently characterized biochemically (91). They are structurally homologuous to COWPs and TgOWP3 localizes specifically in the outer layer. In contrast, tyrosine-rich proteins are small molecules that form protein-protein dityrosine crosslinks responsible for the hardening and natural blue autofluorescence of oocysts under UV light (4). Such proteins have been identified in the oocyst and sporocyst walls of the closely related Coccidia *Eimeria* (3), but not in the *Toxoplasma* oocyst which however exhibits the same typical autofluorescence (73).

In conclusion, current models of the surface chemistry of the environmentally resistant stages of *Giardia*, *Cryptosporidium* and *Toxoplasma* strongly suggest a wall coverage made of complex polymeric matrices. This coverage determines the charge and hydrophobic characteristics of parasites' surface, which are expected to generate and modulate electrostatic attractive / repulsive interactions with the surrounding particles (Table 1).

PARASITE-PARTICLE INTERACTIONS

Due to their size, shape and electrical charges, it is tempting to predict parasite adhesion following the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory of colloidal stability (26, 72, 114). This theory takes into account the electrostatic repulsion between surface charges, which strongly depends on ionic strength of the surrounding liquid, and electro-dynamic attractions due to London-van der Walls forces. At large distances, repulsion is less important than attraction, resulting in an overall attraction, whereas a repulsive barrier due to the glycocalyx must be overcome to reach irreversible adhesion when the interparticle distance becomes small enough. However, applied to the *Cryptosporidium* oocyst, parasite-silica interaction models do not closely fit with the DVLO theory at separations < 35 nm because of the roughness of the oocyst surface and of the extension of the surface macromolecules from the surface into the electrical double layer (20). Thus, some other forces that are not included in the DLVO approximation may have an important contribution to parasite-particle interactions, notably hydrophobic and steric repulsion forces (19, 20, 23). At the nano scale, adhesion strongly depends on the topography (surface roughness) and on the molecular coverage of the parasite surface by macromolecules creating potential attractive / repulsive forces. Surface properties can be investigated by using several physical methods (111), among which is atomic force microscopy (AFM). AFM gives valuable information about the surface topography by directly allowing its imaging at nm-scale resolution, and allows force measurements in physiological media, with unfixed samples (40, 118). AFM uses a nano-finger, at the extremity of a very soft, several micrometers long spring, to gently delineate the surface (imaging mode), to indent the objects surface by pressing on them allowing to gain measurement of mechanical properties of the objet through its Young modulus (force mode, mechanics) (46, 80) or to probe the adhesion of surface molecules when decorated with suitable haptens and pulling the lever off the surface until all built bridges are broken, allowing to directly quantify the force that those bridges can sustain (force mode, adhesion).

171Different variants of adhesion force measurements have been employed so far in cell biology
172(38, 65, 92-94, 107, 112). This technique is now fast-emerging to study environmental
173pathogens (119). To date, AFM has been employed only to observe the surface topography of
174the oocyst of *Cryptosporidium parvum* and measure its mechanical properties (8, 9, 18, 19).
175AFM images describe a rather rough landscape at the oocyst surface, while the measurements
176of its mechanical properties indicate that it is as hard as siliceous materials (18).
177Any modification of the parasite surface chemistry may promote or hinder adhesion. In lab-
178scale experiments using a radial stagnation point flow system to investigate adhesion kinetics
179of *Cryptosporidium* oocysts and *Giardia* cysts, enzymatic treatments by proteinase K or
180pepsine have been found to seriously damage the outer layer of the parasites (69). While the
181surface glycocalyx of *Cryptosporidium* oocysts prevent their adhesion to quartz surfaces by
182imposing a steric repulsion, proteolytic enzymes that cause such a degradation naturally
183enhance their attachment to the very same surfaces (68, 69, 74). Under conditions close to
184natural field ones, dissolved ions and organic contaminants deeply impact the surface
185properties of the parasites. Dissolved calcium ions in solution tend to apparently diminish
186negative charges at the parasite surface, consequently abolishing repulsion, and thus
187enhancing attachment of *Cryptosporidium* oocysts to sand grain surface (68). Using packed-
188bed beads columns to investigate the behavior of *Cryptosporidium* oocysts in granular porous
189media (as a model for sand), Kim et al. showed that increasing ionic strength of the media
190promotes parasite retention in conjunction with a low velocity of the solution flow (62). It has
191been shown, as one may have expected, that parasites exhibit variation in their zeta potential
192when suspended in water-based solutions differing by their conductivity, pH and dissolved
193organic carbon concentrations. For instance, *Toxoplasma* oocysts are negatively charged and
194tend not to aggregate to other particles in freshwater solutions (ζ = -16.16 mV) while their
195global charge becomes near to neutral in higher-ionic-strength solutions that mimic the

conditions encountered in estuarine and seawater (ζ = -1.84 and -2.81 mV respectively), thus leading to efficient parasite aggregation with other particles in these environments (101). According to different studies, the oocyst of *Cryptosporidium parvum* has an isoelectric point of 2.2-3.3 at which electrostatic repulsion forces are abolished (6, 19, 30, 53). In contrast, when placed in presence of dissolved compounds from natural organic matters, parasites exhibit an absolute increase of their negative charges and of their hydrophobicity, possibly due to the adsorption of clays, humic and fulvic acids onto their surface. This may thus enhance transport rather than parasite sedimentation (24, 81, 82, 88, 101).

SURFACE INTERACTIONS DRIVE THE TRANSPORT AND SURVIVAL OF PARASITIC PROTOZOA

At the field-scale, such interactions critically affect the behavior of these pathogens and their distribution in terrestrial and aquatic environments. Transport of parasitic protozoa in soil follows the colloid filtration theory suggesting that the size of these microorganisms control to a large extend their transport in granular media (45). However, the theory does not take into account the surface characteristics of the parasites, their viability state, and their reversible interactions with soil grain surfaces, which promote or inhibit the terrestrial transport of parasitic protozoa (97). Soil physicochemical properties e.g. mineralogy, natural organic matter content, and pH critically affect the parasite-particle interactions and the mobilization behavior of *Cryptosporidium* oocysts (43, 52, 79 81, 82). Spatial dissemination of excreted parasites depends also on local hydrodynamic forces and occurs mainly by leaching, typically following heavy rainfalls, leading to the possible entering of the parasites in waters (39, 84). It has been shown that vegetated wetlands may efficiently retain parasites while degraded habitats promote pathogen pollution of waters with great impacts on humans and animals (100, 106).

The ability of waterborne *Giardia* cysts and *Cryptosporidium* oocysts to settle contributes to either their transport in waters or their retention in sediments. Their respective settling velocity has been determined following the Stokes law, taking into account the parasite diameter and its specific gravity (Table 1). The settling velocity of unattached parasites is relatively low (e.g. 0.35 $\mu m.s^{-1}$ for *Cryptosporidium*) (78), however, when attached to particles, the very same parasites settle faster (~1.3 $\mu m.s^{-1}$) mainly due to an increase of the apparent diameter of the objects and of their specific gravity (49, 78). These parasites are likely to be associated to fecal or soil particles before entering rivers or water reservoirs, so settling may occur more or less efficiently depending on the size of particles, suggesting that some particle-attached parasites may travel along rivers (35). Also, aggregation of the parasites with particles does not preclude neither their survival in water beds for months nor their re-distribution from sediments due to local water turbulences (99). This latter phenomenon may cause recurrent parasitic contaminations as observed in certain surface waters used for drinking (85, 98, 99). Overall, one must consider that such interactions could be responsible for the amazing persistence of infective parasites in water and solid matrices, in conjunction with variations of local physical and chemical conditions (63).

If recent investigations on the molecular coverage and surface forces of *Cryptosporidium*, *Giardia* and *Toxoplasma* parasites have provided important information on the parasite behavior at different spatial and time scales, they have also enlightened some technical limits when working with protozoan cysts and oocysts. In particular, parasites used for imaging, force-based and transport experiments should be carefully purified and stored in order to prevent any modification or loss of their macromolecular coverage caused by chemical agents or by disparities in parasite's populations (6). As a consequence, bleach-sterilized, formalin-treated or heat-inactivated parasites may exhibit modified surface properties and are therefore not suitable for such interaction experiments (9, 42, 68, 69, 88). To overcome this drawback

and because of the biohazard risks associated with the manipulation of resistant parasites, some authors have proposed to use surrogate microspheres and they have been successfully employed for transport experiments. Typically, these substrates are fluorescent glass or latex beads that are designed or decorated to mimic the size and surface properties of the targeted parasites (48). These surrogates allow a relatively good prediction of the behavior of the parasites in waters and soils, including their removal by granular porous media or their adhesion to vegetated strips (24, 81, 82, 100, 101). Interestingly, divergent behaviors have been observed between the surrogate microspheres and the parasites they mimic under particular conditions of ionic strength and organic contamination close to natural field ones. This behavior could be linked to the existence of subtle differences in their respective surface chemistry (48, 82).

IMPLICATIONS FOR NATURAL RESOURCE MANAGEMENT

It has been well demonstrated that the monitoring of protozoan parasites in complex matrices as well as their removal and/or inactivation may be greatly impaired due to their interactions with organic and inorganic contaminants (5, 31). For instance, these interactions critically affect the recovery rates of waterborne cysts and oocysts along the different steps of the process. Sampling surface waters may be problematic because the particle-attached parasites likely settle through gravity faster than free parasites in water bed and thus may not be sampled (98, 99). More importantly, aggregation of parasites impacts their purification when using immunomagnetic separation (IMS) techniques or floatation on dense solutions in case of high organic contamination (15, 31, 66, 71, 113). Parasite-particle complexes exhibit a greater specific gravity and cannot be readily separated by floatation (55, 90, 98) whereas particles can also mask the antigenic sites at the parasite surface, thus hampering the antigen recognition by specific antibodies one can use for IMS or immunofluorescence techniques.

The use of dispersant solutions, chelating agents, detergents or biosurfactants is not always successful in order to prevent unwanted parasite-particle interactions during sample processing (54, 75). Furthermore, even the surface of purified parasites may be still coated with divalent cations and organic substances (humic and fulvic acids) that may interfere with downstream applications such as polymerase chain reaction, which usually give useful information on the species, viability and genetic type of the detected parasites (41, 58, 102).

Interestingly, interactions have a dual role in promoting or preventing parasite removal and/or inactivation depending on treatment methods. Water industrials take advantage of these surface properties in order to remove the parasites from raw waters by using coagulant agents such as aluminum-based salts, iron-based salts or organic polymers. Such agents enhance the aggregation of parasites with other particles, allowing the flocculation of the newly formed complexes and their removal following controlled settling (5). Another significant contribution of the particular surface properties relies on interactions that may occur between parasites and sand during their transport through granular porous media in water treatments, allowing parasite retention on sand filters (62, 110). In contrast, unwanted parasite-particle interactions are clearly detrimental when using physical or chemical disinfectants. UV has been shown to inactivate *Cryptosporidium* and *Giardia* at doses commonly applied by water industrials (>40 mJ/cm²) (51), while for *Toxoplasma* a complete and reliable inactivation has been reported in some studies but not others (33, 116). Parasites entrapped in soft mollusk tissues or attached to sediments may not be totally inactivated at these doses (10, 105). Also, the success of chemical-based inactivation processes may also be compromised for such chlorine and ozone-resistant microorganisms (5, 33, 117). The presence of organic and inorganic compounds associated to parasites in water may require the use of higher ozone doses to achieve parasite inactivation, which may lead to the formation of potentially harmful by-products such as bromates (115). In a similar way, *Cryptosporidium* oocysts and *Giardia*

cysts entrapped in pipe-wall biofilms survive to the concentrations of free chlorine usually used in drinking water systems (2, 50). The capacity of the *Toxoplasma* oocyst to interact with biofilms has not been investigated so far. Such investigations would be of great interest since the implication of biofilm cannot be ruled out for elucidating recurrent cases of waterborne toxoplasmosis in several areas (60).

Modeling the fate and transport of parasitic protozoa may offer a valuable tool for risk assessment. Microbial pathogen modeling basically incorporates specific information about microbial dynamic in conjunction with soil composition, hydrological dynamic, climatic conditions, vegetation, and land management (35). It has been well demonstrated that mathematical models for bacteria are not suitable for predicting the fate and transport of parasitic protozoa (17, 28, 35). In particular, fecal indicators fail to approach water contamination by *Cryptosporidium* and *Giardia* parasites because of the ability of oocysts and cysts to interact and aggregate reversibly with other particles compared to other microorganisms (35, 49). Several specific models have been successfully used for estimating protozoan loads in watersheds in some particular contexts (17, 28, 35). To our knowledge, no such models exist for *Toxoplasma* oocysts at a large scale mainly because too few information is available on their transport properties, survival and prevalence throughout the environment (31, 60, 100). Modeling parasitic protozoa incorporates therefore several major challenges, among which exact parasite surface characterization, the determination of inactivation rate, and improvements in the separation and molecular methods that are used to detect and characterize them in complex matrices. The first point is especially crucial when studying what happens following inactivation processes, in order to assess divergent aggregation and settling behaviors observed between viable and non-viable parasites.

CONCLUSIONS AND FUTURE PERSPECTIVES

The circulation of *Giardia*, *Cryptosporidium* and *Toxoplasma* parasites in the different environmental sources leading to potential human infections strongly depends on how the parasites interact with their surrounding media, mainly other organic and inorganic particles. Hydrophobic, steric and electrostatic attractive/repulsive forces created by the polymeric coverage of the parasite surface greatly enhance or hinder parasite adhesion following the environmental physicochemical factors. They contribute to parasite adhesion to natural organic matters, promoting thus their retention in soils or increasing their deposition kinetic in waterbeds. Consequently, such interactions make parasite fluxes hard to predict on a large scale, affecting therefore the management of resources at risk of microbial pollution.

An accurate characterization of parasite-particle interactions clearly requires additional information on the topography and on the molecular composition of the outer surface of the cyst/oocyst wall. The combination of powerful microscopic and spectroscopic techniques, such as fluorescence microscopy, AFM and/or Raman scattering microscopy, may give new insights on the biochemical nature and arrangement of surface macromolecules as well as the adhesive and mechanical properties of the robust wall (27, 86). To date, only the surface of the oocyst of *Cryptosporidium parvum* has been mapped in terms of force and mechanics, and most efforts should focused now on the surface of other medically-important *Cryptosporidium* species, *Giardia* cysts and *Toxoplasma* oocysts and on the interaction forces that might result from the micro- and nano- structures of their coats.

ACKNOWLEDGEMENTS

This work was supported by the French National Research Agency (grant ANR-09-ALIA-009) and Aix-Marseille University (Préciput 2011 program). PHP is currently supported by ANR JCJC DissecTion program.

345**REFERENCES**

346

3471. **Abudalo R. A., J. N. Ryan, R. W. Harvey, D. W. Metge, and L. Landkamer.** 2010.
348 Influence of organic matter on the transport of *Cryptosporidium parvum* oocysts in a ferric
349 oxyhydroxide-coated quartz sand saturated porous medium. Water Res. **44:**1104-1113.

350

3512. **Angles, M. L., J. P. Chandy, P. T. Cox, I. H. Fisher, and M. R. Warnecke.** 2007.
352 Implications of biofilm-associated waterborne *Cryptosporidium* oocysts for the water
353 industry. Trends Parasitol. **23:**352-356.

354

3553. **Belli, S. I., N. C. Smith, and D. J. Ferguson.** 2006. The coccidian oocyst: a tough nut to
356 crack! Trends Parasitol. **22:**416-423.

357

3584. **Belli, S. I., M. G. Wallach, C. Luxford, M. J. Davies, and N. C. Smith.** 2003. Roles of
359 tyrosine-rich precursor glycoproteins and dityrosine- and 3,4-dihydroxyphenylalanine-
360 mediated protein cross-linking in development of the oocyst wall in the coccidian parasite
361 *Eimeria maxima*. Eukaryot. Cell **2:**456-464.

362

3635. **Betancourt, W. Q., and J. B. Rose.** 2004. Drinking water treatment processes for
364 removal of *Cryptosporidium* and *Giardia*. Vet. Parasitol. **126:**219-234.

365

3666. **Brush, C. F., M. F. Walter, L. J. Anguish, and W. C. Ghiorse.** 1998. Influence of
367 pretreatment and experimental conditions on electrophoretic mobility and hydrophobicity
368 of *Cryptosporidium parvum* oocysts. Appl. Environ. Microbiol. **64:**4439-4445.

369

3707. **Butkus M. A., J. T. Bays, and M. P. Labare.** 2003. Influence of surface characteristics
371 on the stability of *Cryptosporidium parvum* oocysts. Appl. Environ. Microbiol. **69:**3819-
372 3825.
373
3748. **Byrd, T. L, and J. Y. Walz.** 2005. Interaction force profiles between *Cryptosporidium*
375 *parvum* oocysts and silica surfaces. Environ. Sci. Technol. **39:**9574-9582.
376
3779. **Byrd, T. L, and J. Y. Walz.** 2007. Investigation of the interaction force between
378 *Cryptosporidium parvum* oocysts and solid surfaces. Langmuir **23:**7475-7483.
379
38010. **Cantwell, R. E., and R. Hofmann.** 2011. Ultraviolet absorption properties of suspended
381 particulate matter in untreated surface waters. Water Res. **45:**1322-1328.
382
38311. **Chalmers, R. M., and A. P. Davies.** 2010. Minireview: clinical cryptosporidiosis. Exp.
384 Parasitol. **124:**138-146.
385
38612. **Chatterjee, A., S. Banerjee, M. Steffen, R. M. O'Connor, H. D. Ward, P. W. Robbins,**
387 **and J. Samuelson.** 2010. Evidence for mucin-like glycoproteins that tether sporozoites of
388 *Cryptosporidium parvum* to the inner surface of the oocyst wall. Eukaryot. Cell **9:**84-96.
389
39013. **Chatterjee, A., A. Carpentieri, D. M. Ratner, E. Bullitt, C. E. Costello, P. W.**
391 **Robbins, and J. Samuelson.** 2010. *Giardia* cyst wall protein 1 is a lectin that binds to
392 curled fibrils of the GalNAc homopolymer. PLoS Pathogens **6:**e1001059.
393

Chávez-Munguía, C., R. Cedillo-Rivera, and A. Martínez-Palomo. 2004. The ultrastructure of the cyst wall of *Giardia lamblia*. J. Eukaryot. Microbiol. **51:**220-226.

Chesnot, T., and J. Schwartzbrod. 2004. Quantitative and qualitative comparison of density-based purification methods for detection of *Cryptosporidium* oocysts in turbid environmental matrices. J. Microbiol. Methods **58:**375-386.

Chiu, P. W., Y. C. Huang, Y. J. Pan, C. H. Wang, and C. H. Sun. 2010. A novel family of cyst proteins with epidermal growth factor repeats in *Giardia lamblia*. PLoS Negl. Trop. Dis. 2010 **4:**e677.

Coffey, R., E. Cummins, V. O. Flaherty, and M. Cormican. 2010. Analysis of the soil and water assessment tool (SWAT) to model *Cryptosporidium* in surface water sources. Biosystems Engineering **106:**303-314.

Considine, R. F., D. R. Dixon, and C. J. Drummond. 2000. Laterally-resolved force microscopy of biological microspheres - oocysts of *Cryptosporidium parvum*. Langmuir **16:**1323-1330.

Considine, R. F., D. R. Dixon, and C. J. Drummond. 2002. *Oocysts of Cryptosporidium parvum* and model sand surfaces in aqueous solutions: an atomic force microscope (AFM) study. Water Res. **36:**3421-3428.

Considine, R. F., C. J. Drummond, and D. R. Dixon. 2001. Force of interaction between a biocolloid and an inorganic oxide: complexity of surface deformation, roughness, and brushlike behavior. Langmuir **17:**6325-6335.

Dai, X. and J. Boll. 2003. Evaluation of attachment of *Cryptosporidium parvum* and *Giardia lamblia* to soil particles. J. Environ. Qual. **32:**296-304.

Dai, X., and J. Boll. 2006. Settling velocity of *Cryptosporidium parvum* and *Giardia lamblia*. Water Res. **40:**1321-1325.

Dai, X., J. Boll, M. E. Hayes, and D. E. Aston. 2004. Adhesion of *Cryptosporidium parvum* and *Giardia lamblia* to solid surfaces: the role of surface charge and hydrophobicity. Colloids Surf. B Biointerfaces **34:**259-263.

Dai, X., and R. M. Hozalski. 2003. Evaluation of microspheres as surrogates for *Cryptosporidium parvum* oocysts in filtration experiments. Environ. Sci. Technol. **37:**1037-1042.

Davids, B. J., D. S. Reiner, S. R. Birkeland, S. P. Preheim, M. J. Cipriano, A. G. McArthur, and F. D. Gillin. 2006. A new family of giardial cysteine-rich non-VSP protein genes and a novel cyst protein. PLoS One **1:**e44.

Derjaguin, B. V., and L. D. Landau. 1941. Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes. Acta Physicochim. URSS **14:**733-762.

442

44327. **de Souza, W. and G. M. Rocha.** 2011. Atomic force microscopy: a tool to analyze the
444 structural organization of pathogenic protozoa. Trends Parasitol. **27:**160-167.
445

44628. **Dorner, S. M., P. M. Huck, and R. M. Slawson.** 2004. Estimating potential
447 environmental loadings of *Cryptosporidium* spp. and *Campylobacter* spp. from livestock
448 in the Grand River watershed, Ontario, Canada. Environ. Sci. Technol. **38:**3370-3380.
449

45029. **Dorny, P., N. Praet, N. Deckers, and S. Gabriel.** 2009. Emerging food-borne parasites.
451 Vet. Parasitol. **163:**196-206.
452

45330. **Drozd, C., and J. Schwartzbrod.** 1996. Hydrophobic and electrostatic cell surface
454 properties of *Cryptosporidium parvum*. Appl. Environ. Microbiol. **62:**1227-1232.
455

45631. **Dumètre, A., and M. L. Dardé.** 2003. How to detect *Toxoplasma gondii* oocysts in
457 environmental samples? FEMS Microbiol. Rev. **27:**651-661.
458

45932. **Dumètre, A., and M. L. Dardé.** 2004. Purification of *Toxoplasma gondii* oocysts by
460 cesium chloride gradient. J. Microbiol. Methods **56:**427-430.
461

46233. **Dumètre, A., C. Le Bras, M. Baffet, P. Meneceur, J. P. Dubey, F. Derouin, J. P.
463 Duguet, M. Joyeux, and L. Moulin.** 2008. Effects of ozone and ultraviolet radiation
464 treatments on the infectivity of *Toxoplasma gondii* oocysts. Vet. Parasitol. **153:**209-213.
465

466**Fayer, R., J. P. Dubey, and D. S. Lindsay.** 2004. Zoonotic protozoa: from land to sea.
467 Trends Parasitol. **20:**531-536.
468
469**Ferguson, C., A. M. de Roda Husman, N. Altavilla, D. Deere, and N. Ashbolt.** 2003.
470 Fate and transport of surface water pathogens in watersheds. Fate and transport of surface
471 water pathogens in watersheds. Crit. Rev. Environ. Sci. Technol. **33:**299-361.
472
473**Ferguson, D. J., A. Birch-Andersen, J. C. Siim, and W. M. Hutchison.** 1979.
474 Ultrastructural studies on the sporulation of oocysts of *Toxoplasma gondii*. III. Formation
475 of the sporozoites within the sporocysts. Acta Pathol. Microbiol. Scand. B **87:**253-260.
476
477**Ferguson, D. J., S. Brecht, and D. Soldati.** 2000. The microneme protein MIC4, or an
478 MIC4-like protein, is expressed within the macrogamete and associated with oocyst wall
479 formation in *Toxoplasma gondii*. Int. J. Parasitol. **30:**1203-1209.
480
481**Fierro, F. A., A. Taubenberger, P. H. Puech, G. Ehninger, M. Bornhauser, D. J.**
482 **Muller, and T. Illmer.** 2008. BCR/ABL expression of myeloid progenitors increases beta
483 1-integrin mediated adhesion to stromal cells. J. Mol. Biol. **377:**1082-1093.
484
485**Forslund, A., B. Markussen, L. Toenner-Klank, T. B. Bech, O. S. Jacobsen, and A.**
486 **Dalsgaard.** 2011. Leaching of *Cryptosporidium parvum* oocysts, *Escherichia coli*, and a
487 *Salmonella enterica* serovar *Typhimurium* bacteriophage through intact soil cores
488 following surface application and injection of slurry. Appl. Environ. Microbiol. **77:**8129-
489 8138.
490

49140. **Franz, C. M., and P. H. Puech.** 2008. Atomic force microscopy: a versatile tool for
492 studying cell morphology, adhesion and mechanics source. Cell. Mol. Bioeng. **1:**289-300.
493
49441. **Frostegård, A., S. Courtois, V. Ramisse, S. Clerc, D. Bernillon, F. Le Gall, P.**
495 **Jeannin, X. Nesme, and P. Simonet P.** 1999. Quantification of bias related to the
496 extraction of DNA directly from soils. Appl. Environ. Microbiol. **65:**5409-5420.
497
49842. **Gao, X., and J. Chorover.** 2009. In-situ monitoring of *Cryptosporidium parvum* oocyst
499 surface adhesion using ATR-FTIR spectroscopy. Colloids Surf. B Biointerfaces **71:**169-
500 176.
501
50243. **Gao, X., and J. Chorover.** 2011. Amphiphile disruption of pathogen attachment at the
503 hematite (α-Fe2O3)-water interface. Langmuir **27:**5936-5943.
504
50544. **Gottig, N., E. V. Elías, R. Quiroga, M. J. Nores, A. J. Solari, M. C. Touz, and H. D.**
506 **Luján.** 2006. Active and passive mechanisms drive secretory granule biogenesis during
507 differentiation of the intestinal parasite *Giardia lamblia*. J. Biol. Chem. **281:**18156-18166.
508
50945. **Gupta V., W. P. Johnson, P. Shafieian, H. Ryu, A. Alum, M. Abbaszadegan, S. A.**
510 **Hubbs, and T. Rauch-Williams.** 2009. Riverbank filtration: comparison of pilot scale
511 transport with theory. Environ. Sci. Technol. **43:**669-676.
512
51346. **Hampoelz, B., Y. Azou-Gros, R. Fabre, O. Markova, P. H. Puech, and T. Lecuit.**
514 2011. Microtubule-induced nuclear envelope fluctuations control chromatin dynamics in
515 Drosophila embryos. Development In press.

516

51747. **Harris, J. R., and F. Petry.** 1999. *Cryptosporidium parvum* structural components of the
518 oocyst wall. J. Parasitol. **85:**839-849.

519

52048. **Harvey, R. W., D. Metge, R. Sheets, and J. Jasperse.** 2011. Fluorescent microspheres
521 as surrogates in evaluating the efficacy of riverbank filtration for removing
522 *Cryptosporidium parvum* oocysts and other pathogens. NATO Science for Peace and
523 Security Series C: Environmental Security 81-96.

524

52549. **Hawkins, P. R., P. Swanson, M. Warnecke, S. R. Shanker, and C. Nicholson.** 2000.
526 Understanding the fate of *Cryptosporidium* and *Giardia* in storage reservoirs: a legacy of
527 Sydney's water contamination incident. Aqua **49:**289-306.

528

52950. **Helmi, K., S. Skraber, C. Gantzer, R. Willame, L. Hoffmann, and H. M. Cauchie.**
530 2008. Interactions of *Cryptosporidium parvum*, *Giardia lamblia*, vaccinal poliovirus type
531 1, and bacteriophages phiX174 and MS2 with a drinking water biofilm and a wastewater
532 biofilm. Appl. Environ. Microbiol. **74:**2079-2088.

533

53451. **Hijnen, W. A., E. F. Beerendonk, and G. J. Medema.** 2006. Inactivation credit of UV
535 radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. Water Res.
536 **40:**3-22.

537

53852. **Hijnen, W. A., A. J. Brouwer-Hanzens, K. J. Charles, and G. J. Medema.** 2005.
539 Transport of MS2 phage, *Escherichia coli*, *Clostridium perfringens*, *Cryptosporidium*

parvum, and *Giardia intestinalis* in a gravel and a sandy soil. Environ Sci. Technol. **39:**7860-7868.

53. **Hsu, B. M., and C. Huang.** 2002. Influence of ionic strength and pH on hydrophobicity and zeta potential of *Giardia* and *Crytosporidium*. Colloids Surf. A **201:**201-206.

54. **Inoue, M., S. K. Rai, T. Oda, K. Kimura, M. Nakanishi, H. Hotta, and S. Uga.** 2003. A new filter-eluting solution that facilitates improved recovery of *Cryptosporidium* oocysts from water. J. Microbiol. Methods **55:**679-686.

55. **Inoue, M., S. Uga, T. Oda, S. K. Rai, G. Vesey, and H. Hotta.** 2006. Changes of physical and biochemical properties of *Cryptosporidium* oocysts with various storage conditions. Water Res. **40:**881-886.

56. **Janjaroen, D., Y. Liu, M. S. Kuhlenschmidt, T. B. Kuhlenschmidt, and T. H. Nguyen.** 2010. Role of divalent cations on deposition of *Cryptosporidium parvum* oocysts on natural organic matter surfaces. Environ. Sci. Technol. **44:**4519-4524.

57. **Jenkins, M. B., B. S. Eaglesham, L. C. Anthony, S. C. Kachlany, D. D. Bowman, and W. C. Ghiorse.** 2010. Significance of wall structure, macromolecular composition, and surface polymers to the survival and transport of *Cryptosporidium parvum* oocysts. Appl. Environ. Microbiol. **76:**1926–1934.

Jiang, J., K. A. Alderisio, A. Singh, and L. Xiao. 2005. Development of procedures for direct extraction of *Cryptosporidium* DNA from water concentrates and for relief of PCR inhibitors. Appl. Environ. Microbiol. **71:**1135-1141.

Jones, J. L., V. Dargelas, J. Roberts, C. Press, J. S. Remington, and J. G. Montoya. 2009. Risk factors for *Toxoplasma gondii* infection in the United States. Clin. Infect. Dis. **49:**878-884.

Jones, J. L., and J. P. Dubey. 2010. Waterborne toxoplasmosis--recent developments. Exp. Parasitol. **124:**10-25.

Karanis, P., C. Kourenti, and H. Smith. 2007. Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. J. Water Health **5:**1-38.

Kim, H. N., S. L. Walker, and S. A. Bradford. 2010. Coupled factors influencing the transport and retention of *Cryptosporidium parvum* oocysts in saturated porous media. Water Res. **44:**1213-1223.

King, B. J., and P. T. Monis. 2007. Critical processes affecting *Cryptosporidium* oocyst survival in the environment. Parasitology **134:**309-323.

Konrad, C., C. Spycher, and A. B. Hehl. 2010. Selective condensation drives partitioning and sequential secretion of cyst wall proteins in differentiating *Giardia lamblia*. PLoS Pathog. **6:**e1000835.

Krieg, M., Y. Arboleda-Estudillo, P. H. Puech, J. Käfer, F. Graner, D. J. Müller, and C. P. Heisenberg. 2008. Tensile forces govern germ-layer organization in zebrafish. Nature Cell Biol. **10:**429-436.

Kuczynska, E., and D. R. Shelton. 1999. Method for detection and enumeration of *Cryptosporidium parvum* oocysts in feces, manures, and soils. Appl. Environ. Microbiol. **65:**2820-2826.

Kuznar, Z. A., and M. Elimelech. 2004. Adhesion kinetics of viable *Cryptosporidium parvum* oocysts to quartz surfaces. Environ. Sci. Technol. **38:**6839-6845.

Kuznar, Z. A., and M. Elimelech. 2005. Role of surface proteins in the deposition kinetics of *Cryptosporidium parvum* oocysts. Langmuir **21:**710-716.

Kuznar, Z. A., and M. Elimelech. 2006. *Cryptosporidium* oocyst surface macromolecules significantly hinder oocyst attachment. Environ. Sci. Technol. **40:**1837-1842.

Lauwaet, T., B. J. Davids, D. S. Reiner, and F. D. Gillin. 2007. Encystation of *Giardia lamblia*: a model for other parasites. Curr. Opin. Microbiol. **10:**554-559.

Lélu, M., E. Gilot-Fromont, D. Aubert, A. Richaume, E. Afonso, E. Dupuis, C. Gotteland, F. Marnef, M. L. Poulle, A. Dumètre, P. Thulliez, M. L. Dardé, and I. Villena. 2011. Development of a sensitive method for *Toxoplasma gondii* oocyst extraction in soil. Vet. Parasitol. **183:**59-67.

613

61472. **Liang, Y., N. Hilal, P. Langston, and V. Starov.** 2007. Interaction forces between
615 colloidal particles in liquid: theory and experiment. Adv. Colloid Interface Sci. **134-135:**
616 151-166.
617

61873. **Lindquist, H. D., J. W. Bennett, J. D. Hester, M. W. Ware, J. P. Dubey, and W. V.**
619 **Everson.** 2003. Autofluorescence of *Toxoplasma gondii* and related coccidian oocysts. J.
620 Parasitol. 89:865-867.
621

62274. **Liu, Y., M. S. Kuhlenschmidt, T. B. Kuhlenschmidt, and T. H. Nguyen.** 2010.
623 Composition and conformation of *Cryptosporidium parvum* oocyst wall surface
624 macromolecules and their effect on adhesion kinetics of oocysts on quartz surface.
625 Biomacromolecules **11:**2109-2115.
626

62775. **Macarisin, D., G. Bauchan, and R. Fayer.** 2010. *Spinacia oleracea* L. leaf stomata
628 harboring *Cryptosporidium parvum* oocysts: a potential threat to food safety. Appl.
629 Environ. Microbiol. **76:**555-559.
630

63176. **Mai, K., P. A. Sharman, R. A. Walker, M. Katrib, D. De Souza, M. J. McConville,**
632 **M. G. Wallach, S. I. Belli, D. J. Ferguson, and N. C. Smith.** 2009. Oocyst wall
633 formation and composition in coccidian parasites. Mem. Inst. Oswaldo Cruz **104:**281-289.
634

63577. **Maubon, D., D. Ajzenberg, M. P. Brenier-Pinchart, M. L. Dardé, and H. Pelloux.**
636 2008. What are the respective host and parasite contributions to toxoplasmosis? Trends
637 Parasitol. **24:**299-303.

638

63978. **Medema, G. J., F. M. Schets, P. F. Teunis, and A. H. Havelaar.** 1998. Sedimentation
640 of free and attached *Cryptosporidium* oocysts and *Giardia* cysts in water. Appl. Environ.
641 Microbiol. **64:**4460-4466.
642

64379. **Milosavljevic, N., C. Duranton, N. Djerbi, P. H. Puech, P. Gounon, D. Lagadic-**
644 **Gossmann, M. T. Dimanche-Boitrel, C. Rauch, M. Tauc, L. Counillon, and M. Poët.**
645 2010. Nongenomic effects of cisplatin: acute inhibition of mechanosensitive transporters
646 and channels without actin remodeling. Cancer Res. **70:**7514-7522.
647

64880. **Metge, D. W., R. W. Harvey, G. R. Aiken, R. Anders, G. Lincoln, J. Jasperse, and M.**
649 **C. Hill.** 2011. Effects of sediment-associated extractable metals, degree of sediment grain
650 sorting, and dissolved organic carbon upon *Cryptosporidium parvum* removal and
651 transport within riverbank filtration sediments, Sonoma County, California. Environ Sci
652 Technol. **45:**5587-5595.
653

65481. **Mohanram, A., C. Ray, R. W. Harvey, D. W. Metge, J. N. Ryan, J. Chorover, and D.**
655 **D. Eberl.** 2010. Comparison of transport and attachment behaviors of *Cryptosporidium*
656 *parvum* oocysts and oocyst-sized microspheres being advected through three
657 minerologically different granular porous media. Water Res. **44:**5334-5344.
658

65982. **Mohanram, A., C. Ray, D. W. Metge, L. B. Barber, J. N. Ryan, and R. W. Harvey.**
660 2011. Effect of dissolved organic carbon on the transport and attachment behaviors of
661 *Cryptosporidium parvum* oocysts and carboxylate-modified microspheres advected

through temperate humic and tropical volcanic agricultural soil. Environ. Sci. Technol. In press.

Moitinho, M. L. R., M. Bertoli, T. A. Guedes, and C. S. Ferreira. 1999. Influence of refrigeration and formalin on the floatability of *Giardia duodenalis* cysts. Mem. Inst. Oswaldo Cruz **94:**571-574.

Mons, C., A. Dumètre S. Gosselin, C. Galliot, and L. Moulin. 2009. Monitoring of *Cryptosporidium* and *Giardia* river contamination in Paris area. Water Res. **43:**211-217.

Montoya, J. G., and O. Liesenfeld. 2004. Toxoplasmosis. Lancet **363:**1965-1976.

Murugkar, S., C. L. Evans, X. S. Xie, and H. Anis. 2009. Chemically specific imaging of *Cryptosporidium* oocysts using coherent anti-Stokes Raman scattering (CARS) microscopy. J. Microsc. **233:**244-250.

Nanduri, J. S., S. Williams, T. Aji, and T. P. Flanigan. 1999. Characterization of an immunogenic glycocalyx on the surfaces of *Cryptosporidium parvum* oocysts and sporozoites. Infect. Immun. **67:**2022–2024.

Ongerth, J. E., and J. P. Pecoraro. 1996. Electrophoretic mobility of *Cryptosporidium* oocysts and *Giardia* cysts. J. Environ. Eng-ASCE **122:**228.

Petry, F. 2004. Structural analysis of *Cryptosporidium parvum*. Microsc. Microanal. **10:** 586-601.

687

68890. **Poitras, C., J. Fatisson, and N. Tufenkji.** 2009. Real-time microgravimetric
689 quantification of *Cryptosporidium parvum* in the presence of potential interferents. Water
690 Res. **43:**2631-2638.

691

69291. **Possenti, A., S. Cherchi, L. Bertuccini, E. Pozio, J. P. Dubey, and F. Spano.** 2010.
693 Molecular characterisation of a novel family of cysteine-rich proteins of *Toxoplasma*
694 *gondii* and ultrastructural evidence of oocyst wall localisation. Int. J. Parasitol. **40:**1639-
695 1649.

696

69792. **Puech, P. H., D. Nevoltris, P. Robert, L. Limozin, C. Boyer, and P. Bongrand.** 2011.
698 Force measurements of TCR / pMHC recognition at T cell surface. PLoS One **6:**e22344..

699

70093. **Puech, P.H., K. Poole, D. Knebel, and D. J. Muller.** 2006. A new technical approach to
701 quantify cell-cell adhesion forces by AFM. Ultramicroscopy **106:**637-644.

702

70394. **Puech, P. H., A. Taubenberger, F. Ulrich, M. Krieg, D. J. Muller, and C. P.**
704 **Heisenberg.** 2005. Measuring cell adhesion forces of primary gastrulating cells from
705 zebrafish using atomic force microscopy. J. Cell Sci. **118:**4199-4206.

706

70795. **Robertson, L. J.** 2007. The potential for marine bivalve shellfish to act as transmission
708 vehicles for outbreaks of protozoan infections in humans: a review. Int. J. Food Microbiol.
709 **120:**201-216.

710

71196. **Rose, J. B., and T. R. Sliko.** 1999. *Giardia*, *Cryptosporidium*, and *Cyclospora* and their
712 impact on foods: a review. J. Food Prot. **62:**1059-1070.
713
71497. **Santamaría, J., M. D. Quinonez-Diaz, L. Lemond, R. G. Arnold, D. Quanrud, C.**
715 **Gerba, and M. L. Brusseau.** 2011. Transport of *Cryptosporidium parvum* oocysts in
716 sandy soil: Impact of length scale. J Environ Monit. in press.
717
71898. **Searcy, K. E., A. I. Packman, E. R. Atwill, and T. Harter.** 2005. Association of
719 *Cryptosporidium parvum* with suspended particles: impact on oocyst sedimentation. Appl.
720 Environ. Microbiol. **71:**1072-1078.
721
72299. **Searcy, K. E., A. I. Packman, E. R. Atwill, and T. Harter.** 2006. Deposition of
723 *Cryptosporidium* oocysts in streambeds. Appl. Environ. Microbiol. **72:**1810-1816.
724
725100. **Shapiro, K., P. A. Conrad, J. A. K. Mazet, W. W. Wallender, W. A. Miller, and J.**
726 **L. Largier.** 2010. Effect of estuarine wetland degradation on transport of *Toxoplasma*
727 *gondii* surrogates from land to sea. Appl. Environ. Microbiol. **76:**6821-6828.
728
729101. **Shapiro, K., J. Largier, J. A. Mazet, W. Bernt, J. R. Ell, A. C. Melli, and P. A.**
730 **Conrad.** 2009. Surface properties of *Toxoplasma gondii* oocysts and surrogate
731 microspheres. Appl. Environ. Microbiol. **75:**1185-1191.
732
733102. **Sluter, S. D., S. Tzipori, and G. Widmer.** 1997. Parameters affecting polymerase
734 chain reaction detection of waterborne *Cryptosporidium parvum* oocysts. Appl. Microbiol.
735 Biotechnol. **48:**325-330.

736

737103. **Spano, F., C. Puri, L. Ranucci, L. Putignani, and A. Crisanti.** 1997. Cloning of the
738 entire COWP gene of *Cryptosporidium parvum* and ultrastructural localization of the
739 protein during sexual parasite development. Parasitology **114:**427-437.

740

741104. **Speer, C. A., S. Clark, and J. P. Dubey.** 1998. Ultrastructure of the oocysts,
742 sporocysts, and sporozoites of *Toxoplasma gondii*. J. Parasitol. **84:**505-512.

743

744105. **Sunnotel, O., W. J. Snelling, N. McDonough, L. Browne, J. E. Moore, J. S.**
745 **Dooley, and C. J. Lowery.** 2007. Effectiveness of standard UV depuration at inactivating
746 *Cryptosporidium parvum* recovered from spiked Pacific oysters (*Crassostrea gigas*).
747 Appl. Environ. Microbiol. **73:**5083-5087.

748

749106. **Tate, K. W., M. D. Pereira, and E. R. Atwill.** 2004. Efficacy of vegetated buffer
750 strips for retaining *Cryptosporidium parvum*. J. Environ. Qual. **33:**2243-2251.

751

752107. **Taubenberger, A., D. A. Cisneros, J. Friedrichs, P. H. Puech, D. J. Muller, and C.**
753 **M. Franz.** 2007. Revealing early steps of α2β1 integrin-mediated adhesion to collagen
754 type I by using single-cell force spectroscopy. Mol. Biol. Cell **18:**1634-1644.

755

756108. **Templeton, T. J., C. A. Lancto, V. Vigdorovich, C. Liu, N. R. London, K. Z.**
757 **Hadsall, and M. S. Abrahamsen.** 2004 The *Cryptosporidium* oocyst wall protein is a
758 member of a multigene family and has a homolog in *Toxoplasma*. Infect. Immun. **72:**980-
759 987.

760

761109. **Thompson, R. C.** 2004. The zoonotic significance and molecular epidemiology of
762 *Giardia* and giardiasis. Vet. Parasitol. **126:**15-35.
763
764110. **Tufenkji, N., D. R. Nixon, R. Considine, and C. J. Drummond.** 2006. Multi-scale
765 *Cryptosporidium*/sand interactions in water treatments. Water Res. **40:**3315-3331.
766
767111. **Ubbink, J., and P. Schär-Zammaretti.** 2005. Probing bacterial interactions:
768 integrated approaches combining atomic force microscopy, electron microscopy and
769 biophysical techniques. Micron **36:**293-320.
770
771112. **Ulrich, F., M. Krieg, E. M. Schötz, V. Link, I. Castanon, V. Schnabel, A.**
772 **Taubenberger, D. Mueller, P. H. Puech, and C. P. Heisenberg.** 2005. Wnt11 functions
773 in gastrulation by controlling cell cohesion through Rab5c and E-cadherin Dev. Cell
774 **9:**555-564.
775
776113. **USEPA**. 2005. Method 1623: *Cryptosporidium* and *Giardia* in water by
777 filtration/IMS/FA. EPA-815-R-05-002.
778
779114. **Vitte, J., A. M. Benoliel, A. Pierres, and P. Bongrand.** 2004. Is there a predictable
780 relationship between surface physical-chemical properties and cell behaviour at the
781 interface? Eur. Cell. Mater. **7:**52-63.
782
783115. **von Gunten, U.** 2003. Ozonation of drinking water: part II. Disinfection and by-
784 product formation in presence of bromide, iodide or chlorine. Water Res. **37:**1469-1487.
785

116. **Wainwright, K. E., M. Lagunas-Solar, M. A. Miller, B. C. Barr, I. A. Gardner, C. Pina, A. C. Melli, A. E. Packham, N. Zeng, T. Truong, and P. A. Conrad.** 2007. Physical inactivation of *Toxoplasma gondii* oocysts in water. Appl. Environ. Microbiol. **73:**5663-5666.

117. **Wainwright, K. E., M. A. Miller, B. C. Barr, I. A. Gardner, A. C. Melli, T. Essert, A. E. Packham, T. Truong, M. Lagunas-Solar, and P. A. Conrad.** 2007. Chemical inactivation of *Toxoplasma gondii* oocysts in water. J. Parasitol. **93:**925-931.

118. **Webb, H. K., V. K. Truong, J. Hasan, R. J. Crawford, and E. P. Ivanova.** 2011. Physico-mechanical characterisation of cells using atomic force microscopy - current research and methodologies. J. Microbiol. Methods **86:**131-139.

119. **Wright, C.J., M.K. Shah, L.C. Powell, and I. Armstrong.** 2010. Application of AFM from microbial cell to biofilm. Scanning **32:**134-149.

120. **Young, P. L., and S. J. Komisar.** 2005. Impacts of viability and purification on the specific gravity of *Cryptosporidium* oocysts. Water Res. **39:**3349-3359.

121. **Young, P. L., and S. J. Komisar.** 2005. Settling behavior of unpurified *Cryptosporidium* oocysts in laboratory settling columns. Environ. Sci. Technol. **39:**2636-2644.

TABLE 1: Physicochemical characteristics of the environmentally resistant stages of *Giardia duodenalis*, *Cryptosporidium* spp. and *Toxoplasma gondii*.

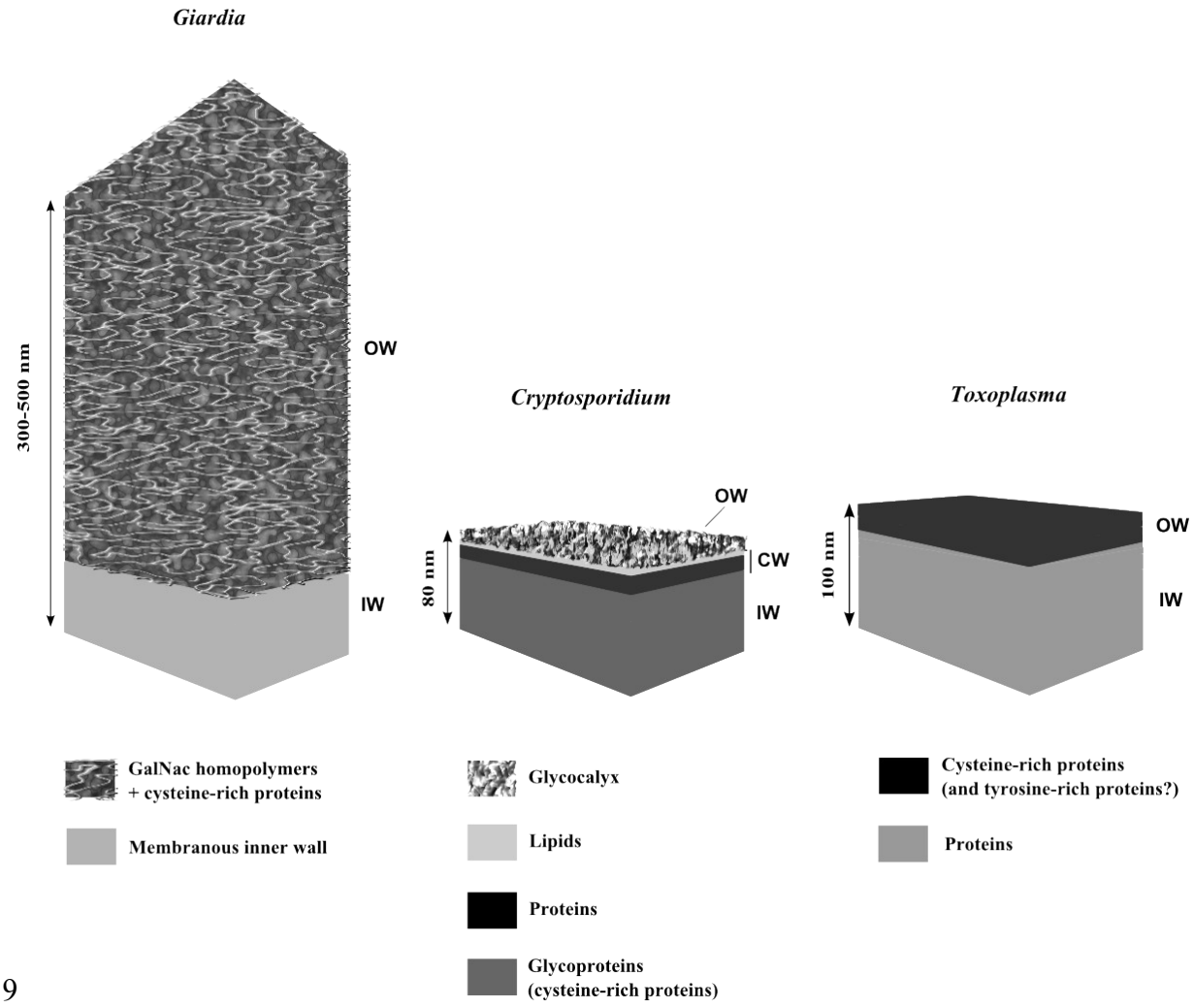
	Giardia duodenalis cyst	*Cryptosporidium* spp. oocyst	*Toxoplasma gondii* oocyst	References
Size (µm)	7-10 x 5	3.8-6.3 x 4.6-8.4[a]	10 x 12	11, 60, 109
Wall				
Thickness (nm)	300-500	50-70	~100	14, 37, 57
Number of layers	2	3	2	
Outer wall thickness (nm)	250	8	30	
Surface biochemistry	Matrix of filamentous GalNAc homopolymer and Cys-rich proteins	Glucose-rich glycocalyx	Possible polymeric cross-links of Cys-/ Tyr-rich proteins	14, 37, 57, 64, 76, 103
Zeta potential (mV)	-33.5 in distilled H_2O at pH 6.4	-25.0 in deionized H_2O (conductivity 83.9 µS.cm^{-1} at pH 6.5)	-43.7 in ultrapure H_2O (conductivity 4 µS.cm^{-1} at pH 6.7)	30, 88, 101
Specific gravity	1.013-1.117	1.009-1.08	1.050-1.100	22, 31, 55, 78, 83, 120
Settling velocity (µm.s^{-1})	0.84-1.4	0.35-1.31	Not reported	22, 78, 121

[a] depending on species..

LEGEND TO FIGURE 1: Schematic drawing of the different walls of the *Giardia* cyst, and *Cryptosporidium* and *Toxoplasma* oocysts. OW: outer wall; CW: central wall; IW : inner wall.

817FIGURE 1

818



819

820